# A NEW MYCOCALICIUM ON SCARRED SEQUOIA IN CALIFORNIA

Lee Bonar
Department of Botany, University of California, Berkeley 94720

Observations and collections have been made through many years of a discomycetous fungus that grows as a mat on an exudate from the surface of exposed heartwood of living specimens of the big tree, *Sequoiadendron giganteum* (Lindl.) Buchh., and the coast redwood, *Sequoia sempervirens* (Lamb.) Endl., in California. The fungus is never found on unscarred trees. It is present on the charred surface of burns and rarely on other large scars where there has been a flow of exudate from the wood.

The exudate flows down over the charred surface in spreading dark sheets or strands. It is watery in the fresh portion, becoming soft and sticky, then hard and brittle, lustrous Hessian Brown (R) to black on exposure and drying. The dried portion sometimes forms sheets up to 0.5 cm thick or balls up to 3 cm in diameter. In one instance the exudate streamed down over the burn for 5 m with the fungus growing over the lower 3 m of the area. The exudate usually issues from the more recently formed wood adjacent to the callus and not on the deeper parts of the burns.

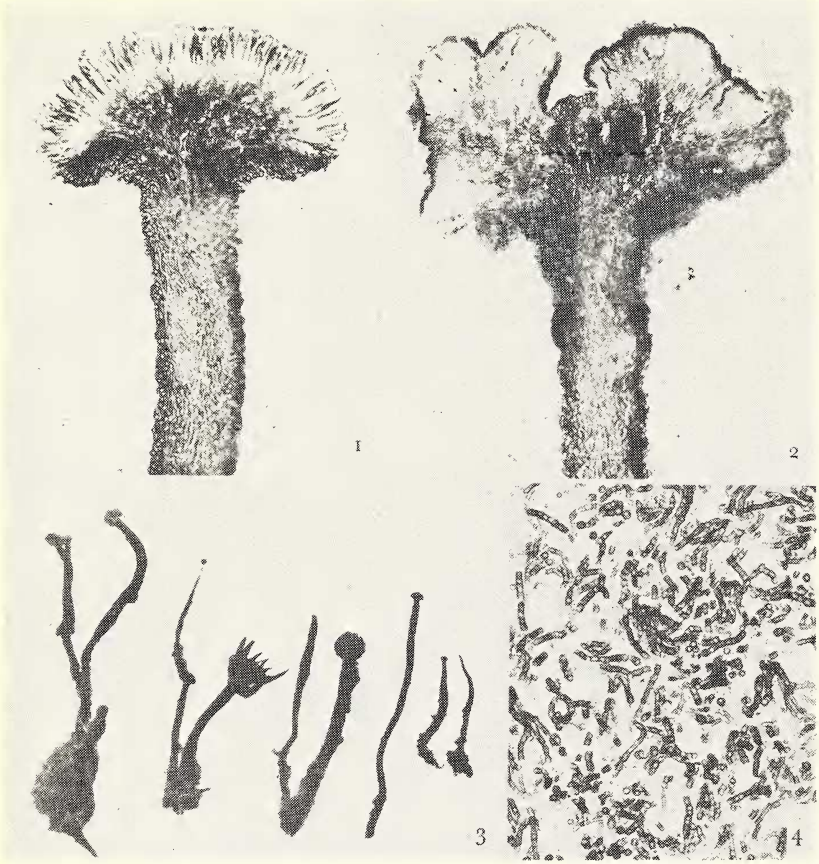
Limited chemical examination of the dried exudate indicated that it is a very complex mixture of organic compounds, mostly water soluble, and very different from the resinous exudates from conifers such as pines and firs.

Surveys of fire-scarred specimens of the big tree were made in three areas:

| | Exudate and fungus present | Exudate and fungus absent |
|---|---|---|
| Sequoia National Park (Giant Forest and Swanee Grove) | 145 | 5 |
| Yosemite National Park (Mariposa Grove) | 35 | 5 |
| Calaveras State Park (Calaveras Grove) | 28 | 8 |
| Total | 208 | 18 |

Many fire-scarred trees of *Pinus, Abies,* and *Libocedrus* were examined in each of the above areas but no growth of the fungus was found.

The fungus has been found less often and less profuse in growth on the coast redwood than on the big tree of the Sierra Nevada. Flow of exudate onto scarred surfaces of the trees was found to be much more limited and growth of the fungus correspondingly less. For example, a survey of 63 fire-scarred trees in Big Basin Redwoods State Park, Santa Cruz Co., California, showed exudate present on 9, visible growth of the fungus on 3 with 1 showing mature apothecia, while 60 of the trees



FIGS. 1–4. *Mycocalicium sequoiae:* 1, median section of apothecium (mazaedium lost in processing) ; 2, median section of lobulate apothecial head, × 82; 3, apothecia dissected from pseudostroma; early stages right, proliferating secondary growth left, × 6; 4, microtome section of pseudostroma showing interlaced hyphae, × 82.

lacked any growth of the fungus. A search in the Cazadero Creek area in Sonoma Co. resulted in finding one tree with exudate and a growth of the fungus 5 × 3 cm in extent.

Approximately 100 fire-scarred trees were examined in Humboldt State Park, Humboldt Co., especially in the vicinity of Weott and Dyerville, and limited growth was found on 3 trees. Two of these occurrences were not on fire scars: scanty growth without fruting, forming a line of growth at the outer margin of the heartwood, where some exudate had appeared, was found on the cut end of a log from a recently felled tree. A second tree, at the roadside, had received a relatively large cut to remove the buttress next to the road. Quite copious exudate flowed from the cut and the fungus had formed a matted growth up to 3 cm thick; this showed some mature apothecia.

Besides scarcity of the exudate, another factor limiting growth of the fungus on the redwood is the growth of superficial molds over the surface of the discomycete mat. The molds frequently form floccose growths over the surface of the mat and apparently inhibit growth and fruiting of the discomycete. Such mold growths were seen in only a few instances on the fungus on the big tree. Growth of such superficial molds is undoubtedly favored by the relatively higher humidity surrounding those fire scars near the ground and commonly in deep shade in the fog-ridden belt occupied by the redwood. Corresponding sites on fire scars of big trees are surrounded through the long dry Sierran summer by less favorable environment for surface mold growth.

## Description of the Fungus

Vegetative growth of the fungus varies from a floccose subiculum (very rare) to a mat-like pseudostroma (Vainio, 1890). The pseudostroma is resupinate and adherent to the charred surface. In extent of growth it varies greatly with moisture present. Examples were found where the pseudostromata covered a few $cm^2$, while some could be measured in $m^2$. The maximum development observed, on an extensive burn on a very large tree in Swanee Creek Grove, Sequoia National Park, measured 6 m at the ground perimeter and spread upward over the burn 0.5 to 1.5 m with tongues of growth extending upward for 2.4 m.

The pseudostroma is commonly 0.5 to 2 cm thick but may be up to 4.5 cm. It is composed of interlaced hyphae (fig. 4) which in younger growth near the edge are abundantly encrusted with crystals of calcium oxalate. The hyphae vary greatly in form and pigmentation. Those of younger portions appear hyaline under the microscope with thickened gelantinized walls. The walls do not stain—hence the spaced arrangement in the figure. In older portions the hyphae are mostly free of crystals, become amber to deep brown in color, and are frequently made up of torulose cells. The hyphae are 7-15 $\mu$ in diameter, with narrow lumina.

Stipitate apothecia develop in large numbers within the pseudostroma and grow to project above the surface of the mat (fig. 5). Stipes first appear as compact bundles of interlaced hyphae. As these elongate toward the surface of the pseudostroma, the outer cells of the cylindrical structure become thick-walled and dark in color. The stipes emerge through the surface of the pseudostroma as minute blackish columns with acute tips, giving the surface a distinctly setose character. There may be as many as 100 of these per $cm^2$. The stipes are 5-6 (4-8) mm long. Many remain as sterile pointed structures. In others, there is a branching of the hyphae within the tip and development of a palisade of hyphal elements to form the hymenium. The outer layer of blackened cells is reflexed to form the exciple (fig. 1), which surrounds a plane to convex hymenium. The surface of the hymenium is Chrysolite Green


FIGS. 5–7. *Mycocalicium sequoiae;* 5, surface view of apothecia in nature; 6 mature apothecia from culture on wood block, vegetative growth scanty and floccose; 7, side view of apothecia in position in pseudostroma, × 15.

(R) and is farinose when dry due to the numerous green amorphous crystals attached to the surface. The apothecial heads are 0.5–1.5 mm in diameter. Stipes are frequently branched at various levels. Branching most commonly occurs near the tip, resulting in development of 2-20 contiguous convexities forming a lobulate head on a common stipe (fig. 2). Branching may occur at lower levels, each branch forming an apothecial head (fig. 7).

The stipitate apothecia are frequently found completely buried in the pseudostroma due to continued surface growth of the pseudostroma. These may show a second stipe developed as a proliferation through the hymenium of the first (fig. 3) or, more commonly, new stipes develop and grow through to the new surface.

Asci appear first near the center of the hymenial area and successively toward the margin as the surface expands and becomes more convex. The asci are inoperculate, cylindric-clavate, short stipitate, 50–70 × 6–10 $\mu$, 8-spored, with the apical portion thick-walled. The walls of the asci stain blue with iodine. The spores are 1-celled, smooth, brown, uniseriate to biseriate in the ascus and 6–8 × 4–5 $\mu$. Paraphyses are abundant, branched, colorless, with swollen tips 3–5 $\mu$ in diameter.

The walls and stipes of the asci gelatinize early, leaving the ascospores in linear groups of eight embedded in a gelatinous matrix and then extruded to the surface of the disk to form a mazaedium (Acharius, 1817). Acharius defined mazaedium as the superficial amorphous layer over the surface of the hymenium. Smith (1921), Dennis (1960), and Ainsworth (1961) define it as a type of fruit body or apothecium.

The production of asci is long-continued, new ones forming in areas where earlier ones have gelatinized and have been extruded to the surface.

No spermogonia were found as are described for some species of *Calicium* and related genera.

## Cultural Studies

Germination tests showed a high percentage of viability in spores of material that had been held in a dry condition for as long as a year's time. Single ascospore cultures were grown on a number of common types of agar media and on extracts of *Sequoia* wood in agar. Maximum growth was a mycelial colony 2–5 mm in diameter at the end of 1 month at room temperature.

Blocks of heartwood of *Sequoiadendron giganteum* 15 cm long by 2.5 cm diameter and found by microscopic examination to be free of any fungus hyphae were soaked in water and sterilized by autoclaving in glass culture vessels with water to keep the lower ends of the blocks moist. Young colonies from single ascospores were placed on the end of the blocks. Such cultures were held at 10°C in dark, at room temperature in light and in dark, and in a daylight chamber at outdoor temperature. Growth occurred under all these conditions. It varied from light-brown floccose cushions to a delicate scarcely visible subiculum on the surface of portions of the blocks, the growth becoming darker with age. Least growth was in the chambers held at 10°C. Best growth was at outdoor temperature. Cultures held at room temperature showed an intermediate rate of growth. No marked difference was observed in vegetative growth in light and in darkness. No conidia were observed in any of the cultures.

Initial development of stipitate apothecia appeared in cultures in the outdoor chamber at the end of 4 months and these matured by the end

of 5 to 6 months (fig. 6). Cultures held at room temperature did not develop apothecia in either light or dark. Those held at 10°C for 5 months were sterile. Part of these were then moved to the outdoor chamber and apothecia developed after 3 additional months. Apothecia developed in culture chambers showed typical asci and spores. Vegetative growth was not the compact pseudostroma found in nature but at most a loose cottony felt (fig. 6).

## Mycelium in Wood

Blocks of heartwood up to 5 cm thick were cut out from under burned areas in standing trees where the surface was covered by an extensive growth of the fungus. The wood appeared to be sound with no macroscopic evidence of decay. Microscopic examination of this wood showed occasional hyphae in the lumina of the wood cells, but there was no evidence of any appreciable destruction of the cell walls. The blocks of wood used for culturing the fungus were split and examined at intervals up to 1 year from the time the cultures were started, having been maintained in a moist condition. By the end of 3 months from the time of inoculation, hyphae were found within the cells throughout the length of the blocks. The hyphae ramified through the wood cells both longitudinally and transversely. The transverse hyphae commonly passed through pits and occasionally penetrated directly through the walls. Blocks that had been subjected to the action of the fungus for 1 year showed some discoloration, but no other evidence of change. Microscopic examination of these showed only slight corrosion of the cell walls. The evidence indicates that this fungus is capable of very slow digestion of wood when compared with the rate of action of other common wood-rotting fungi.

Field studies indicate that the fungus grows primarily on the exudate from the wood, this providing both the water and food material. Wood underlying areas bearing a heavy growth of the surface pseudostroma—which is certainly several years old—is sound in appearance and shows only very limited development of hyphae in the wood tissues. Kimmey and Lytle (1952) concur, saying, "Sterile black fructifications of an ascomycete were occasionally found growing in association with exudate from rift cracks in exposed heartwood but were not associated with decay."

## Classification

The characters of the apothecium place this fungus in the Caliciaceae. This family is usually classified under the lichens and its members usually consist of a fungus-algal complex. Various authors have, however, described species under this family as lacking any algal component. They have frequently placed such species in the same genera with typical lichen species. Vainio (1890) established the genus *Mycocalicium* for species lacking gonidia and having one-celled, brown ascospores. Reinke

(1895) proposed the Protocaliciaceae as a sub-family under the Caliciaceae for those organisms that were non-lichen in nature and cited *Mycocalicium* and *Mycoconiocybe* as generic names for non-lichen members of the genera *Calicium* and *Coniocybe*. Vainio (1927) lists some seven genera of gonidia-free organisms under the Caliciaceae and gives descriptions for five species of *Mycocalicium*.

Various other workers have not accepted these proposals. Rehm (1896) lists seven genera and numerous species of this group of Discomycetes that do not have algal components. He states that the majority of the species in the family are true lichens and no generic distinction is made between the lichen and non-lichen members. He lists *Mycocalicium* as a synonym under *Calicium*. Schneider (1897) used the name *Mycocalicium* for typical lichens with one-celled ascospores as a distinction from *Calicium* with two-celled ascospores. The name was used in the same sense by Nearing (1962). Keissler (1938) lists all the gonidia-free segregates as synonyms under the regular lichen genera having gonidia, and discusses the difficulty of being able to know for certain that the lack of gonidia may not be merely a fortuitous circumstance. Arnaud (1931) presented the Caliciaceae as a complex of diverse elements drawn from the lichens, Sphaeriaceae, Perisporiaceae, Hysteriaceae, etc. It is very difficult to characterize clearly such a mixture. I prefer to consider the family as a more restricted group.

Clements and Shear (1931) divided the genera of the Caliciaceae into two groups: one saprophytic and non-lichen, the other forming typical lichen thalli with algae.

Studies, including the complete life cycle in pure culture, have proved that our fungus is not a lichen. The form of the apothecia, the manner of maturation, and the characteristics of the ascospores agree with those of the genus *Mycocalicium* as established by Vainio. Vegetative development and habitat are highly distinctive and lead to the conclusion that this is an underscribed species of *Mycocalicium*.

**Mycocalicium sequoiae** Bonar, sp. nov. Pars vegetativa corallina uvidaque ex subiculo hyphae intertextae vel ex pseudostromate expanso crassitudine usque ad 4.5 cm constans, in sinectute atrata rimosaque; apothecia super superficium extendentia; stipes teres, simplex vel ramosus, 4.8 mm longus; apice acuto demum capita turbinata diam. 0.5–1.5 mm formante; apothecia primaria demum pseudostromate obvoluta inde evolvent plura; asci inoperculati, cylindro-clavati, 8-spori, evanescentes, 50–70 × 6–10 $\mu$, parietes iodino tingentes; gelatina sporaeque mazaedium in sicco crystalla irridia obtectae formantes; ascosporae 1-cellulae, ellipsoideae, fuscae, 6–8 × 4–5 $\mu$, in asco uni-vel biseriatae; paraphyses ramosae; sporae asexuales deficientes.

Habitat. On exudate from exposed heartwood of living sequoias in California.

Holotype. On *Sequoiadendron giganteum,* Crescent Meadow, Sequoia

National Park, Tulare C.o, California, *Lee Bonar* (UC 1403569-holotype), July 1, 1935.

Collections studied. In addition to the holotype, 12 collections on *S. giganteum* from Calaveras, Mariposa, and Tulare counties, and 4 collections on *S. sempervirens* from Humboldt, Sonoma and Santa Cruz counties (all UC) were studied.

I am indebted to various persons for collections and aid in this study, especially to Doris Brenneman for help in the critical laboratory studies, to Rimo Bacigalupi for the Latin description, and to Victor Duran and A. A. Blaker for photographs.

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## NOTES AND NEWS

Wallace Roy Ernst, 1928–1971.—Wallace Ernst, Curator of Botany in the Smithsonian Institution died of cancer in Washington, D.C., on October 8th, 1971, after an illness of about nine months. In addition to his Smithsonian curatorship, he held a professorship in absentia at the University of Kansas, and was for a number of years a valuable member of the editorial board of Madroño. Dr. Ernst was a recognized authority in the systematics of the Papaveraceae and an accomplished worker in the field of floral morphology. He received his first two degrees at the University of California at Los Angeles and his doctorate at Stanford University. An appreciation and biography will appear in Madroño in the near future.

—J. H. T.